

**Herbal formulation of *Azadirachta indica* and *Stevia rebaudiana* and its anti-inflammatory and anti-diabetic activity.**

UNDER PEER REVIEW

## ABSTRACT

### INTRODUCTION:

*Azadirachta indica*, a natural herb also called *Neem*, and its seed extract has various medicinal uses. *Neem* ~~is also~~ believed to possess notable insecticidal and pesticidal activities. On the other hand, *Stevia rebaudiana* extract has alpha amylase glycosidase inhibition inhibitory activity, which contrasts with the traditional wheat bread. This last plant can also ~~*Stevia rebaudiana*~~ regulate blood glucose levels, and prevent hypertension and caries.

Formatted: Font: Italic

AIM: This study aimed to analyse the anti-diabetic and anti-inflammatory activities of *Azadirachta indica* and *stevia rebaudiana*.

MATERIALS AND METHODS: 2.5gms ~~5 g of from~~ *Azadirachta indica* and *Stevia rebaudiana* plants extract were added mixed within 100 mL ~~millilitre~~ of distilled water and were boiled. The final extract was then filtered using whatmann ~~Whatman~~ filter paper. Spearman correlation analysis was done by SPSS. Positive correlation analysis level of significance was set as  $r=1$ .

RESULTS: The anti-diabetic activity and anti-inflammatory activities of *Azadirachta indica* and *Stevia rebaudiana* extracts ~~showed~~ positive spearman correlations analysis ( $r = 1$ ). Both activities happen in a dose-dependent manner, being higher with the ~~corresponding to the~~ absorbance with respective rise in concentration of the extract. Anti-inflammatory activity and Anti-diabetic activity of *Stevia rebaudiana* and *Azadirachta indica* showed positive correlation with increased of the concentration followed by rise and of in the absorbance values at different concentrations.

Comment [ACAG1]: Exactly 1?

### CONCLUSION:-

Through this study, it was possible to conclude ~~concludeed~~ that *Azadirachta indica* and *Stevia rebaudiana* have great anti-inflammatory and anti-diabetic activity ~~activities~~ and can be used as a possible alternative drug for attenuating ~~treating~~ inflammation and preventing ~~diabetic complications~~ patients.

Keywords: ~~*Stevia rebaudiana*, *Azadirachta indica*, herbal~~ Herbal formulation, Anti ~~Anti~~ Inflammatory, Antidiabetic, Green synthesis.

Running title: Anti-inflammatory and anti-diabetic activity of *Stevia rebaudiana* and *Azadirachta indica*

## INTRODUCTION

*Azadirachta indica* (*Neem*) is a natural herb also called Indian lilac or Neem tree. *Azadirachta indica* is a natural herb. This plant and its seeds exhibit several medicinal uses. *Neem* is also believed for its insecticidal and pesticide activities. Additionally, *Neem* oil is a typical and effective pest repellent against sand fleas and mosquitoes. *Neem* can also control and repel moths, termites, ticks and fleas. They are also added to cattle grain to repel parasites and also to help control pest growth. *Neem* acts as a free radical neutralizer by enacting its active role as an anti-oxidant agent. *Neem* has also antimicrobial effects, being effective against several bacteria, viruses and fungi (1). Focusing on *Stevia rebaudiana*, this is a plant species belonging to the Asteraceae (Sunflower) family of the genus *Stevia* (2). *Stevia* leaves contain alkaloids, flavonoids, chlorophylls, xanthophyll, amino acid and lipids. *Stevia* can grow to a height of 1–2 feet, and its leaves and flowers boost its flavours. *Stevia rebaudiana* belongs to a plant family of sweet herbs native to South America, Brazil, Southeast Asia and China. The plant extract has beneficial health effects, namely anti-hypertensive, anti-human rotavirus and anti-hyperglycaemia effects (3).

Diabetes mellitus is characterized by a rise in glucose level due to irregular metabolism of carbohydrates. 25% of the world population were affected from diabetes, and its appearance happens in both developing and developed countries (4,5). Blood glucose levels are maintained by the human body within the constrictive range by glucagon and insulin. Type I diabetes is the inability to metabolize glucose with increased uptake by fat tissue and muscles (6). Causes of Type II diabetes are mainly derived from environmental and dietary habits. Complications of diabetes involve hyperglycaemia, hyperosmolar state, diabetic neuropathy, hypoglycaemia, angiopathy and nephropathy (7). *Azadirachta indica* is reported to have medicinal values, including antidiabetic properties. Study as reported by Raajshree et al., on using in vivo diabetic murine model against Streptozotocin induced diabetes

Formatted: Font: Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

~~in albino rat~~ diabetic murine and rat models (8) Therefore, *A. indica* and *Stevia* is largely investigated for the biochemical parameters important for owing to their abilities to control ~~ling~~ diabetes, ~~source of free~~ scavenging free radicals and reactive species activity and anti-inflammatory activity effects. In fact, The antioxidant activity of *S. rebaudiana* extract was already showed to be effective with a rise in concentrations in a dose-dependent manner. The mode of anti-inflammatory activity of the phenolic ~~compound~~ component is believed to show ~~an~~ be associated with their scavenging capacity effective radical scavenging activity through the donation of hydrogen atoms (9,10).- *Stevia rebaudiana* reduces blood glucose, dental caries and prevents hypertension. Likewise, it has shown ~~an~~ effective anti-viral and anti-bacterial ~~property~~ properties (11). The aim of the present investigation is the preparation of Herbal formulation of *A. indica* and *S. rebaudiana* and to analyse its anti-oxidant and anti-diabetic activities ~~y~~-(12-31);(13-26);(27-31)

#### Materials and methods:

Plant extract preparation:

*Stevia rebaudiana* and *Azadirachta indica* powdered extracts ~~was easily available and~~ were collected from the herbal health care centre ~~from where?~~ (Figure 1) 100 mL of distilled water was added to 2.5 gms of *A. indica* and *Stevia S. rebaudiana* in a beaker and boiled. The solutions were heated for 10-20 minutes in the heating mantle and cooled at room temperature.-(Figure 2)-. The boiled extract was filtered using ~~Whatman~~ filter paper. Random sampling method was done in an unbiased manner. Micropipetting ~~has had~~ to be done with care to avoid manual error. The extract was prepared in Blue lab at Saveetha dental college and hospital, Chennai, ~~country~~. The validation of the extract preparation procedure was done by Nano research experts.

#### Anti-diabetic activity:

##### Reagent and chemicals

- 3,5-dinitrosalicylic acid solution (DNSA) reagent (100 µL)
- 5 mL of solutions of aqua alcoholic *B. diffusa* extracts of different concentrations (10 to 50 µg/mL)

**Positive control:** acarbose

**Negative control:**  $\alpha$ - amylase solution\_(-100  $\mu$ L), starch solution (100  $\mu$ L) [from which company?](#)

UNDER PEER REVIEW

#### **-ALPHA-AMYLASE INHIBITORY ASSAY:**

Bhutkar and Bhise followed the quantifying method with liberated maltose amounts in the experiment to determine Alpha-amylase inhibition. 100µL of solution of alpha amylase- (1U/mL) ~~are were~~ pre-incubated with different concentrations (20µL, 40µL, 60µL, 80µL, 100µL) of the nanoparticles at 37 °C ~~degree celsius~~ for 30 minutes (~~figure~~ Figure 3). 100µL of starch solution was added- (1% w/v-) to- the mixture at 37°C for 10 minutes. 3,5-dinitrosalicylic acid solution (DNSA) reagent 96 ~~millimole-mM~~ of 100 µL was added in order to stop further reaction of the solution and heated for 5 minutes in a water bath. Control and sodium phosphate buffer maintained at -pH value of 6.9. Readings were recorded at 540nm. Acarbose was ~~maintained~~ used as a positive control. The blank is not used here?

~~Formula~~ The formula used for the % inhibition was ~~calculated using~~

$$\% \text{ inhibition} = \frac{C-T}{C} \times 100$$

Where, C= control, T= test sample

#### **Anti-inflammatory activity:**

##### **Reagent and chemicals**

- DPPH in methanol (1 ml of 0.1 mM ) ,
- Herbal formulation of *Azadirachta indica* and *Stevia rebaudiana* (2-10 µg/ml)

**Control group:** Butylated hydroxytoluene

##### **DPPH METHOD (2,2-diphenyl-1-picrylhydrazyl) THIS IS A ANTIOXIDANT ASSAY**

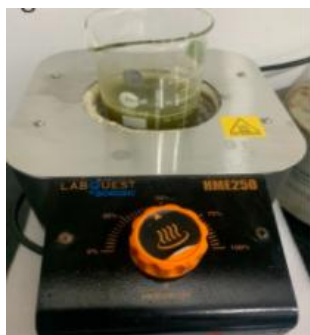
DPPH ~~were was~~ held to test the antioxidant activity of *Azadirachta indica* and *Stevia rebaudiana* based herbal formulations. Diverse concentrations of extracts were added to DPPH ~~of at~~ concentrations ranging from 1 mL of to 0.1 mM in methanol. ~~Therefore, and~~ 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of plant extract (2-10 ~~µg~~ µg/ml) were mixed with 1 mL of 0.1 mM DPPH in methanol and 450µl of Tris HCL buffer of 50mM -(pH 7.4) -were also added- followed by 30 minutes incubation. The absorbances at 517nm were noted to observe the quantity of free radical reduction by DPPH. Butylated hydroxy toluene was the control. (Figure3). The percentage of inhibition was calculated by-

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample} \times 100}{\text{Absorbance of control}}$$

The data obtained are analysed by spearman Rho correlation analysis using (IBM) SPSS 23 version. Positive correlation analysis level of significance was set as  $r=1$ .

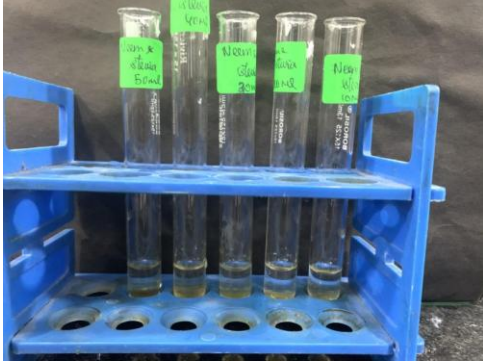


**Figure 1:** Measured 2.5 gmg of *Neem* in a weighing machine.



**Figure 2:** Plant extract boiled at 60 °C degree celsius for 10-20 minutes.

**Formatted:** Font: Bold

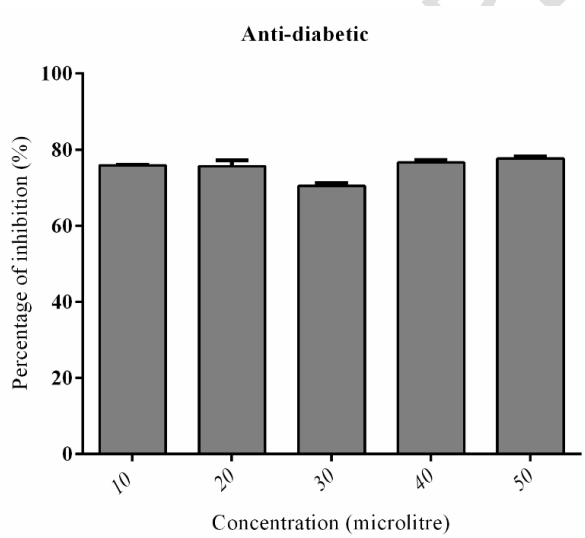


**Figure 3:** Incubation of nanoparticles with alpha amylase solution (100  $\mu\text{L}$ ).

**Comment [ACAG2]:** What nanoparticles? The combination formulation

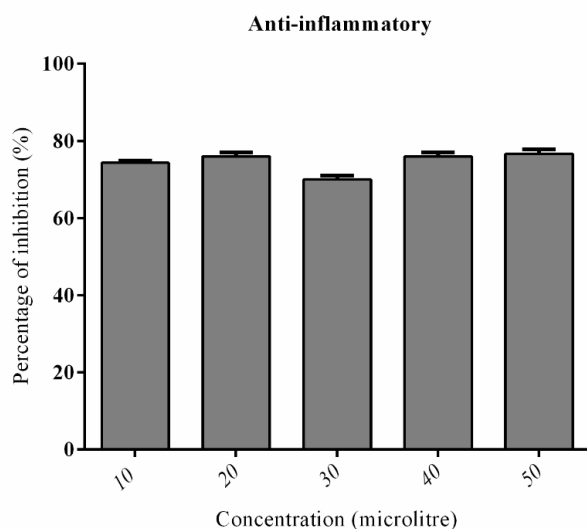
**Formatted:** Font: Bold

## RESULTS



**Figure 4:** Anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* extracts were represented in the above graph. The X-axis represents the concentration of the extract in microlitre and the Y-axis represents the percentage of inhibition. Spearman positive correlation is seen between concentration ( $\mu\text{L}$ ) and antidiabetic activity, being higher with the increasing concentration ( $\mu\text{L}$ ).

**Formatted:** Font: Bold



**Figure 5:** Anti-inflammatory activity of *Azadirachta indica* and *Stevia rebaudiana* extracts were represented in the above graph. The X-axis represents the concentration of the extract in microlitre and the Y-axis represents the absorbance. Spearman-Spearman positive correlation (r=1) was observed with the increase in concentration (µL).

Formatted: Font: Bold

The anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* extracts showed positive Spearman-Spearman correlations (r=1) corresponding to the absorbance with the respective rise in the concentration of the extract (20 µL, 40 µL, 60 µL, 80 µL, 100 µL) (Figure 4). Anti-inflammatory activity of *Azadirachta indica* and *Stevia rebaudiana* showed positive correlations (r=1) with increased concentration followed by rising absorbance values and also shows statistical differences. increased concentration followed by rise in absorbance which is statistically significant.

Graphs on antioxidant activity represented different concentrations (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) in microlitre with absorbance of 0.75, 0.76, 0.71, 0.77, 0.78 at 660 nm. *Azadirachta indica* and *Stevia rebaudiana* extract showed positive anti diabetic activity (r=1)

with increase in concentration with absorbance of 0.075, 0.076, 0.071, 0.077, 0.078 at 540 nm wavelength.

UNDER PEER REVIEW

## DISCUSSION

~~The~~ ~~This~~ study revealed that *Neem* and *Stevia* together possess anti-inflammatory activity and anti-diabetic ~~activity~~ ~~activities~~. It is clear that by increasing the concentration of *Azadirachta indica* and *Stevia rebaudiana* by adding 10  $\mu$ L every time, ~~it increases~~ the percentage of inhibition on both anti-diabetic and anti-inflammatory activity ~~increases~~. Other articles ~~also~~ ~~based on the biological potential of~~ *Azadirachta indica* and *Stevia rebaudiana* ~~also~~ show that ~~it~~ ~~has~~ ~~both~~ ~~plants~~ ~~exhibit~~ antioxidant, cytotoxic effects, anti-hyperlipidemic, anti-bacterial, anti-microbial and hepatoprotective effects (32). Correlation analysis was done to analyse anti-oxidant and anti-diabetic of *Azadirachta indica* and *Stevia rebaudiana* ~~and WHAT?~~. ~~This fact is not surprising and it is in accordance with~~ ~~the~~ study ~~by of~~ ~~satvika~~ ~~Satvika~~ et.al.,. These authors stated that *Neem* and *Aloe vera* synthesized silver nanoparticles have evident antidiabetic activity, ~~namely by~~ ~~it~~ ~~revealed~~ ~~good~~ ~~notorious~~ amylase inhibition (33). ~~Furthermore, an in-~~ ~~vitro~~ study ~~regarding on~~ *Piperlongum* ~~based~~ ~~Silver~~ ~~silver~~ nanoparticles showed an effective anti-inflammatory activity with a maximum inhibition of 81.1% at 20  $\mu$ L ~~microlitre~~ concentration (34). ~~Another study~~ ~~iesy which explains~~ ~~showed the effect of that~~ powdered seed of *Azadirachta indica* ~~which~~ has good anti-diabetic activity (35,36). Previous studies done by Patil et al., ~~already~~ ~~on~~ ~~revealed that~~ *Neem* and *Stevia* ~~revealed~~ ~~possess~~ medicinal properties, like anti-inflammatory, anti-hypertensive and anti-oxidant activity (37) *Ginger oleoresin* ~~combined with their silver nanoparticles mediated silver nanoparticles~~ have effective anti-oxidant and anti-inflammatory activity ~~activities~~ ~~is~~ ~~detected in combination of~~ *Ginger oleoresin* and silver nanoparticle extract. Their activity ~~increase~~ ~~increases~~ ~~by raising the concentration in~~ a dose-dependent manner (38). ~~Additionally,~~ anti-inflammatory activity was ~~also analysed and tested~~ ~~tested~~ ~~against in~~ human blood ~~samples~~ with *Ocimum basilicum* and silver nanoparticles avenues. ~~The obtained promising data can be also considered an added-value for this study against human bodily ailments which add on to its medicinal advantage.~~ Selva priya et.al., have ~~experimented~~ ~~the~~ ~~evaluated~~ ~~the~~ ~~Anti~~ ~~anti~~ inflammatory activity and cytotoxic activity of *Neem* and *Stevia* ~~based~~ herbal extracts. ~~They obtained~~ ~~which~~ ~~revealed~~ ~~the~~ highest absorbance at 50  $\mu$ L ~~microlitre~~ concentration with a maximum inhibition through albumin denaturation assay (39) ~~Anti-inflammatory activity using silver nanoparticles and cumin oil~~ ~~which~~ can be used along with NSAIDs. Hypoinsulinemia and hyperglycaemia leads to diabetes mellitus ~~development~~. The ~~higher~~ flavonoids content of plant extracts ~~stimulate~~ ~~stimulates~~ the beta cells of ~~L~~angerhans to secrete insulin and reduce the

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

glucose level, and in this way, restoring ~~the~~ body weight. The attribution to hyperglycemia, hypoinsulinemia and increased proteolysis leads to the reduction in body weight after STZ-induced DM- (40). The treatment ~~for the~~of diabetic animals with the phenolic-rich plant extracts may result in the improvements of theon body weight ~~because of its high content of flavonoids~~ (41).

Previous study articles on alpha glucosidase and amylase inhibitory activity by *Stevia* plant extract showed inhibitory activity against alpha amylase and glucosidase in hyperglycaemia. (42–44). ~~The Some~~ limitations of our study ~~on anti-inflammatory and anti-diabetic activity of Azadirachta indica and Stevia rebaudiana is its lessare the poor tested~~ concentration and small sample ~~size~~number. ~~Even so, S studies works~~ with increased concentration and a larger sample size can be done. ~~Only~~It is also important to note that deeper in vitro studies are needed before the realization of in-vivo studies with diabetic patients. ~~anti diabetic, and the anti-inflammatory property is analyzed and standardized form of anti-diabetic drug extract of Azadirachta indica and Stevia rebaudiana can be given for patients with diabetes after clinical trials in future.~~

Comment [ACAG3]: Something like that, no?

## CONCLUSION

This study concludes that *Azadirachta indica* and *Stevi rebaudiana* have great anti-inflammatory and anti-diabetic activity and can be used as a possible alternative drug for ~~treating-attenuation,~~ or even treating, inflammatory conditions and preventing diabetic ~~patients~~complications. Even so, ~~f~~Further research experiments have to be done to replace the adverse effects causing drugs with natural plant extract-based medications.

## REFERENCE

1. Cheung T, Nigam P, Owusu-Apenten R. Antioxidant Activity of Curcumin and Neem (*Azadirachta indica*) Powders: Combination Studies with ALA Using MCF-7 Breast Cancer Cells. *Journal of Applied Life Sciences International*. 2016;4(3):1–12.
2. Varghese B, Naithani SC. Desiccation-induced changes in lipid peroxidation, superoxide level and antioxidant enzymes activity in neem (*Azadirachta indica* A. Juss) seeds. *Acta Physiologiae Plantarum*. 2002;24(1):79–87.

3. Sharma Y, Kaushik J, Bala K. Antioxidant Activity of Isolated Protein Mixtures from Seeds of *Azadirachta indica*, *Ocimum sanctum* and *Momordica charantia*. *European Journal of Medicinal Plants*. 2017;19(2):1–10.
4. Arumugam G, Manjula P, Paari N. A review: Anti diabetic medicinal plants used for diabetes mellitus. *Journal of Acute Disease*. 2013;2(3):196–200.
5. Preethikaa S, Brundha MP. Awareness of diabetes mellitus among general population. *Research Journal of Pharmacy and Technology*. 2018;11(5):1825–9.
6. L. ND, Lehninger AL, Nelson DL, Cox MM, University Michael M Cox. *Lehninger Principles of Biochemistry*. Vol. 3. Macmillan; 2005.
7. Rajeshkumar S, Ponnaniakamideen M, Malarkodi C, Malini M, Annadurai G. Microbe-mediated synthesis of antimicrobial semiconductor nanoparticles by marine bacteria. *Journal of Nanostructure in Chemistry*. 2014;4(2):80–98.
8. Raajshree.r K, R KR, Durairaj B. IN VITRO ANTICANCER POTENTIAL OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES FROM THE SEAWEED *TURBINARIA CONOIDES*. *Asian Journal of Pharmaceutical and Clinical Research*. 2018;11(5):127.
9. Bharat P, Sagar R, Sulav R, Ankit P. Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. *African Journal of Biotechnology*. 2015;14(46):3159–63.
10. Brundha MP, Pathmashri VP, Sundari S. Quantitative Changes of Red Blood cells in Cancer Patients under Palliative Radiotherapy-A Retrospective Study. *Research Journal of Pharmacy and Technology*. 2019;12(2):687–92.
11. S N, Nurdiana S. Antidiabetic Activity of *Azadirachta excelsa* Extract on Alloxan Induced Diabetic Rats. *The Open Conference Proceedings Journal*. 2013;4(1):49–52.
12. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer

patients. *Am J Cancer Res.* 2020 Aug 1;10(8):2546–54.

13. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. *Hypertens Res.* 2020 Dec;43(12):1459–61.
14. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in *Streptococcus* sp., using computational approach. *Journal of King Saud University - Science.* 2020 Jan 1;32(1):904–9.
15. Smiline Girija AS. Delineating the Immuno-Dominant Antigenic Vaccine Peptides Against gacS-Sensor Kinase in *Acinetobacter baumannii*: An in silico Investigational Approach. *Front Microbiol.* 2020 Sep 8;11:2078.
16. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University - Science.* 2020 Dec 1;32(8):3380–7.
17. Girija ASS. Fox3+ CD25+ CD4+ T-regulatory cells may transform the nCoV's final destiny to CNS! *J Med Virol* [Internet]. 2020 Sep 3; Available from: <http://dx.doi.org/10.1002/jmv.26482>
18. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: putative interactions, associated gene alterations, and molecular pathways. *Environ Sci Pollut Res Int.* 2021 Jun;28(21):27162–73.
19. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. *Arch Oral Biol.* 2021 Feb;122:105030.
20. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences* [Internet]. 2020;82(2). Available from: <https://www.ijpsonline.com/articles/targeting-nm23h1mediated-inhibition-of-tumour->

metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html

21. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type  $\beta$ -lactamases among *Acinetobacter baumannii* in patients with severe urinary tract infection. *Acta Microbiol Immunol Hung*. 2019 Dec 9;67(1):49–55.
22. Priyadharsini JV, Paramasivam A. RNA editors: key regulators of viral response in cancer patients. *Epigenomics*. 2021 Feb;13(3):165–7.
23. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An in-silico approach. *Acta Virol*. 2020;64(1):93–9.
24. Girija As S, Priyadharsini J V, A P. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI). *Acta Clin Belg*. 2021 Apr;76(2):106–12.
25. Anchana SR, Girija SAS, Gunasekaran S, Priyadharsini VJ. Detection of *csgA* gene in carbapenem-resistant *Acinetobacter baumannii* strains and targeting with *Ocimum sanctum* biocompounds. *Iran J Basic Med Sci*. 2021 May;24(5):690–8.
26. Girija ASS, Shoba G, Priyadharsini JV. Accessing the T-Cell and B-Cell Immuno-Dominant Peptides from *A.baumannii* Biofilm Associated Protein (bap) as Vaccine Candidates: A Computational Approach. *Int J Pept Res Ther*. 2021 Mar 1;27(1):37–45.
27. Arvind P TR, Jain RK. Skeletally anchored forsus fatigue resistant device for correction of Class II malocclusions-A systematic review and meta-analysis. *Orthod Craniofac Res*. 2021 Feb;24(1):52–61.
28. Venugopal A, Vaid N, Bowman SJ. Outstanding, yet redundant? After all, you may be another Choluteca Bridge! *Semin Orthod*. 2021 Mar 1;27(1):53–6.
29. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. *Clin Oral Investig*. 2019 Sep;23(9):3543–50.

Formatted: Portuguese (Portugal)

Formatted: Portuguese (Portugal)

Formatted: Portuguese (Portugal)

Formatted: Portuguese (Portugal)

Field Code Changed

Formatted: Portuguese (Portugal)

30. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. *J Dent Educ.* 2019 Apr;83(4):445–50.
31. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial [Internet]. Vol. 24, *Clinical Oral Investigations.* 2020. p. 3275–80. Available from: <http://dx.doi.org/10.1007/s00784-020-03204-9>
32. Brundha M. Liquid Paraffin as a Rehydrant for Air Dried Buccal Smear. Raipur. 2019 Mar;12(3):1197–200.
33. Jp Y, Yadav JP, Kumar S. Characterization and Antibacterial Activity of Synthesized Silver and Iron Nanoparticles using Aloe vera. *Journal of Nanomedicine & Nanotechnology.* 2016;7(3).
34. Obuli Ganesh Kishore S, R Priyadharshini, S Rajeshkumar, Palati Sinduja, Ant İnflammatory and Antimicrobial Activity of Silver Nanoparticles Synthesized Using Piper Longum, *J Res Med Dent Sci,* 2021, 9(10): 70-76
35. Atangwho IJ, Ebong PE, Eyong EU, Asmawi MZ, Ahmad M. Synergistic antidiabetic activity of *Vernonia amygdalina* and *Azadirachta indica*: Biochemical effects and possible mechanism. *J Ethnopharmacol.* 2016 Dec 4;193(2):725.
36. Vergallo C, Panzarini E, Dini L. High performance liquid chromatographic profiling of antioxidant and antidiabetic flavonoids purified from *Azadirachta indica* (neem) leaf ethanolic extract. *Pure and Applied Chemistry.* 2019;91(10):1631–40.
37. Patil P, Patil S, Mane A, Verma S. Antidiabetic activity of alcoholic extract of Neem (*Azadirachta Indica*) root bark. *National Journal of Physiology, Pharmacy and Pharmacology.* 2013;3(2):142.
38. Swathy S, Roy A, Rajeshkumar S. Anti-inflammatory activity of Ginger oleoresin mediated Silver nanoparticles. *Research Journal of Pharmacy and Technology.* 2020;13(10):4591.

Formatted: Portuguese (Portugal)

Field Code Changed

Formatted: Portuguese (Portugal)

39. Selvapriya, S., Priyadharshini, R., Rajeshkumar, S. and Sinduja, P. (2021) "Anti-Inflammatory and Cytotoxic Effect of Stevia and Neem Based Herbal Formulation", *Journal of Pharmaceutical Research International*, 33(59A), pp. 150-160. doi: 10.9734/jpri/2021/v33i59A34259.
40. Bhuvaneswari P, Krishnakumari S. Nephroprotective effects of ethanolic extract of *Sesamum indicum* seeds (Linn.) in streptozotocin induced diabetic male albino rats. *International Journal of Green Pharmacy (IJGP)*. 2012;6(4):28–42.
41. Zhang F, Feng J, Zhang J, Kang X, Qian D. Quercetin modulates AMPK/SIRT1/NF- $\kappa$ B signaling to inhibit inflammatory/oxidative stress responses in diabetic high fat diet-induced atherosclerosis in the rat carotid artery. *Exp Ther Med*. 2020;20(6):1–1.
42. Al-Massarani SM, El-Gamal AA, Parvez MK, Al-Dosari MS, Al-Said MS, Abdel-Kader MS, et al. New Cytotoxic Seco-Type Triterpene and Labdane-Type Diterpenes from *Nuxia oppositifolia*. *Molecules*. 2017 Mar 2;22(3):130–5.
43. J. C. Ruiz-Ruiz, Y. B. Moguel-Ordoñez, A. J. Matus-Basto & M. R. Segura-Campos. Antidiabetic and antioxidant activity of *Stevia rebaudiana* extracts (Var. Morita) and their incorporation into a potential functional bread. *J Food Sci Technol*. 2015;52(2):7893–903.
44. Ruiz-Ruiz JC, Moguel-Ordoñez YB, Matus-Basto AJ, Segura-Campos MR. Antidiabetic and antioxidant activity of *Stevia rebaudiana* extracts (Var. Morita) and their incorporation into a potential functional bread. *Journal of Food Science and Technology*. 2015;52(12):7894–903.

Formatted: Portuguese (Portugal)

Formatted: Portuguese (Portugal)

Formatted: Portuguese (Portugal)

Field Code Changed

Formatted: Portuguese (Portugal)